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Analyzing aberrantly expressed mRNA profiles in early lung squamous cell carcinoma based on TCGA data

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Abstract: **As one of the most frequent and deadly cancers, the pathological mechanism of lung cancer has yet to be determined, especially in lung squamous cell carcinoma (LUSC). Aberrantly expressed mRNAs are the prominent contributing factor to cancer, which are important to find new biomarkers for early diagnosis and understand the etiology of LUSC. Currently, data of the Cancer Genome Atlas (TCGA) offer the potential to finish these works. This study explored the early expression profile of LUSC-specific mRNA based on a database obtained from TCGA. KEGG analysis showed that P53 signaling pathway, Wnt signaling pathway, TNF signaling pathway, PI3K-Akt signaling and so on have significantly changed in patients with LUSC. Some genes, such as cyclin-dependent kinases 1 (CDK1), minichromosome maintenance complex component 2 (MCM2) and cyclin B1 (CCNB1), are highly expressed in early LUSC, while growth arrest and DNA damage inducible beta (GADD45B), transforming growth factor-β2 (TGFB2) and cell division cycle 14A (CDC14A) and other genes showed low expression, which these genes are enriched in the p53 signaling pathway. The result obtained by KEGG analysis on differentially expressed genes in LUSC tumors are a good indicator of early detection for LUSC, and provide valuable information in future research for clarifying the etiology of LUSC.**

Keywords: Lung squamous cell carcinoma; TCGA; KEGG; p53

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1. Introduction

Lung cancer is the leading of death for cancer patients worldwide, with highest morbidity and mortality[1]. Non-small cell lung cancer accounts for the main type of lung cancer[2], of which lung squamous cell carcinoma (LUSC) has a high degree of malignancy, low clinical cure rate and poor survival rate[3]. This situation is largely due to the lack of in-depth understanding of the molecular pathogenesis of lung squamous cell carcinoma, which makes it impossible to achieve effective targeted therapy. The abnormal regulation of RNA is the essential factor leading to the malignant transformation of normal cells and the formation of tumors[4]. After deep sequencing technology has been applied to the study of lung cancer, the early recognition of the primary tumor and targeting agents of lung adenocarcinoma have made great progress in non-small cell lung cancer while those work on LUSC is relatively delayed.

Therefore, in the present study we explored the early expression profile of LUSC-specific mRNA by analyzing RNA sequence results from 504 LUSC tumors and 46 samples of adjacent nontumorous lung tissues[5]. Differential expressed key genes in the early stage of LUSC can be a good indicator of early detection for LUSC. The research results can provide new clues for clarifying the etiology of LUSC in future.

2. Materials and Methods

2.1. Research Design

All available mRNA expression data were collected from 504 LUSC tumors and 46 adjacent non-tumor lung tissues in TCGA (https://cancergenome.nih.gov/). According to previously reported methods[6], a total of 375 LUSC samples were included in this study. In these data sets, available tumor data were subdivided into early stage group, middle stage group and late stage group, with 40 adjacent non-tumor lung tissues as the control group. Differing from middle stage group, late stage group and the control group, differential expression genes in early stage group were included into following bioinformatics analysis, and detailed information has been described in previous studies.

2.2. Bioinformatics Analysis

Using DAVID (https://david.ncifcrf.gov/), statistically significant differentially expressed mRNAs were examined in Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.kegg.jp/)[7]. Significantly enriched in early LUSC ($P \leq 0.05$ and $FDR \leq 0.05$)[8] were obtained by the pathway enrichment analysis uncovered the metabolic pathways and signal transduction pathways. Some upregulated and downregulated genes were focused.

2.3. Statistical analysis

In this work, significant differences in gene expression were defined by two criteria: false discovery rate ($FDR \leq 0.05$) and fold change (≥ 3). Differences with P values <0.05 were considered statistically significant.

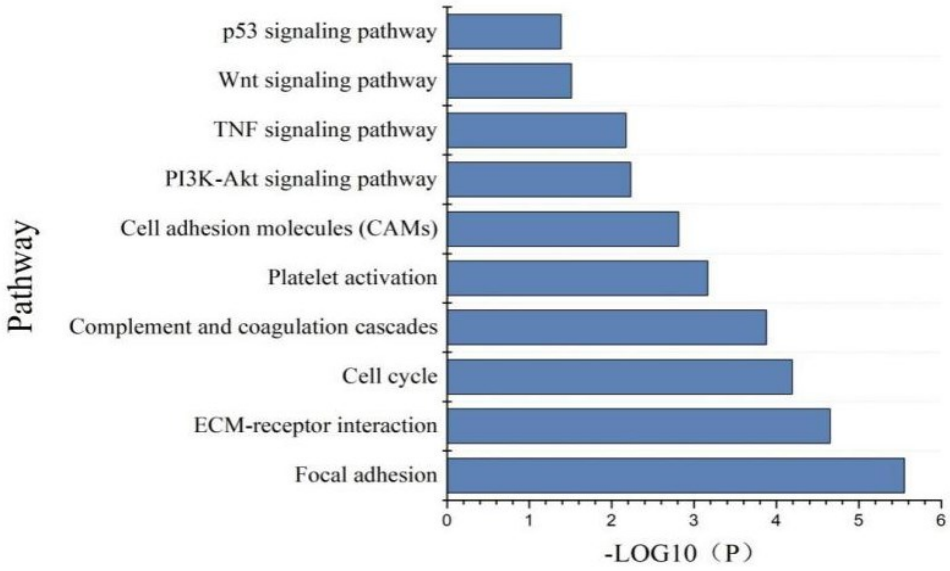

Figure 1. KEGG analysis of differentially expressed genes in early lung squamous carcinoma cells.

3. Results

3.1. Pathway analysis on differentially expressed genes in early LUSC

In our study, data on the differential expression mRNAs in LUSC and adjacent non-tumor lung tissues were obtained from TCGA. Significantly differentially expressed mRNAs in early LUSC were identified, and 8618 differentially expressed genes were obtained. Using DAVID, pathway data analysis showed that the P53 signaling pathway, Wnt signaling pathway, and TNF signaling pathway produced a significant response in patients with LUSC (Figure 1). At the same time, early aberrant signal pathway responses also appeared in other important biological processes including the cell cycle, cell proliferation and adhesion (Figure 1).

3.2. Up-regulated expression key genes in p53 signaling pathway of early LUSC

After KEGG analysis of differentially expressed genes obtained from early LUSC, we found that a total of 62 differentially expressed genes were enriched in the p53 signaling pathway. Among them, 53 up-regulated expression differentially expressed genes were identified including CDK1, CDK2 and CDK4 from cyclin-dependent kinase family, MCM2 and MCM3 from microchromosome maintenance protein family, CCNB1 and CCNB2 cyclin family, etc. The results suggested that abnormal cell growth, chromosome and cell cycle changes were the prominent contributing factor to early LUSC, which contributed to LUSC starting.

3.3. Down-regulated expression key genes in p53 signaling pathway of early LUSC

In addition to 53 up-regulated genes in early LUSC, we also found 9 down-regulated genes in the p53 signaling pathway of early LUSC, such as transforming growth factor-β2 (TGF-β2), cyclin D3 (CCND3), cyclin B3 (CCNB3), cyclin cycle protein 14A (CDC14A), growth arrest and DNA-damage-inducible 45 beta (GADD45B) and so on. The results suggested that some negative effects on cell cycle regulation, immune cell activation and cellular differentiation have formed after normal cells were transformed into LUSC cells.

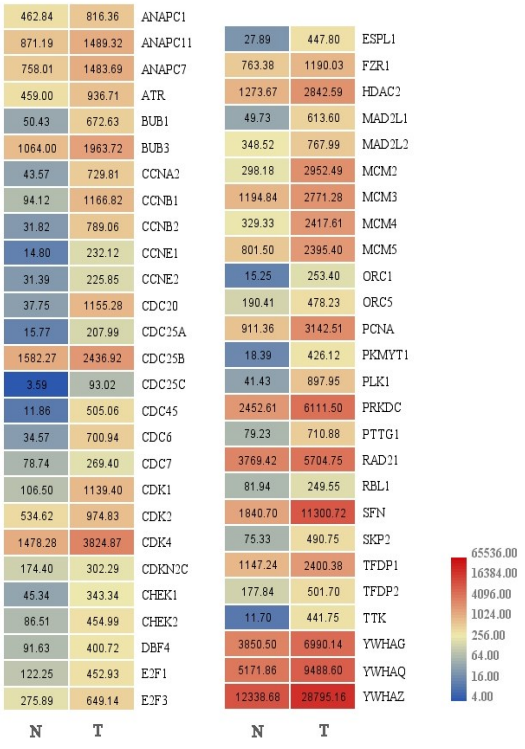

Figure 2. Up-regulated genes enriched in p53 signaling pathway of early LUSC.

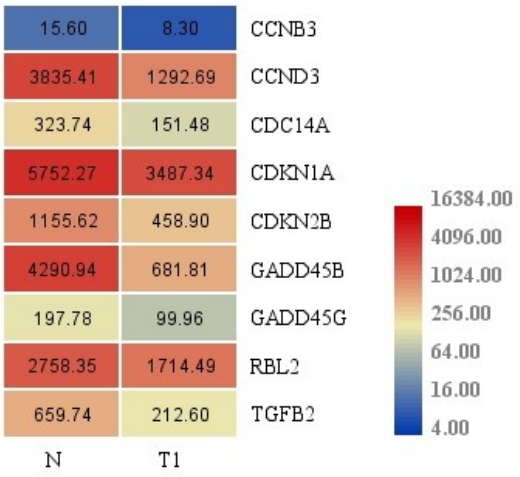

Figure 3. Down-regulated genes enriched in p53 signaling pathway of early LUSC.

4. Discussion

Although there have been some progress on research of lung cancer, the pathological mechanism of LUSC has yet to be determined. As a public funded project, TCGA aims to discover major cancer-causing genomic alterations so as to create a comprehensive "atlas" of cancer genomic profiles[9]. To identify aberrantly expressed mRNA that contributes to early pathologic mechanisms, the updated genomic information of LUSC was analyzed by performing a large number of genomic sequences and comprehensive multidimensional analysis of TCGA datasets in our study. The results showed dynamic changes of LUSC-related genes, and the altered mRNA expression profiles reflected genetic changes and tumor performance in early stage of LUSC.

Compared and screened differences in gene expression in early LUSC and normal tissues based on data from the TCGA database, the KEGG pathway analysis highlighted the dysregulation of mRNA expression which indicated that physiological function of LUSC tissue is significantly different from those in normal tissue. Meanwhile, many genes that were significantly differentially expressed in LUSC were found to be enriched in some important pathways, such as "Wnt signaling pathway", "PI3K-AKT signaling pathway", etc. The activation of Wnt/β-catenin signaling and up regulated Wnt signaling target genes in human non-small cell lung cancer (NSCLC) cells resulted in promoted cell proliferation and invasion[10]. The PI3K-AKT signaling pathway was a key pathway for cancer cell growth, proliferation and migration, and thus invasion of human ovarian cancer cells were inhibited through the inactivation of the PI3K/Akt signaling pathway[11]. These results suggested that abnormal proliferation of early LUSC was associated with abnormal changes in intracellular pathways. In addition to the above pathways, some different expressed genes with high or low expression werw enriched in the "p53 signaling pathway", including high expression genes (CCNB1, CDK2, MCM2, etc.) and low expression genes (TGF-β2, CDKN2A, RBL2, etc.).

Among the highly expressed genes, we found that CDK1, CDK2 and CDK4 were significantly up-regulated in early stage lung squamous cell carcinoma. CDKs can be inhibited by some inhibitors such as p18INK4c to restrain the proliferation and tumorigenesis of mammary luminal progenitor cell[12]. Therefore, the high expression of CDKs in the early stage of lung squamous cell carcinoma revealed that the tumor has enhanced ability in proliferation and differentiation, which may further lead the future aggressiveness of LUSC.

TGF-β, a multifunctional regulator, is associated with cell growth and differentiation[13,14]. Researchers have noticed that TGF-β acts as a tumor suppressor in early stages of tumor[15]. At the same time, we found that TGF-β2 was lowly expressed in early stage of LUSE, which was consistent with its role in the early development of cancer. The result suggested that TGF-β2 and related gene expression can be used as biomarkers for understanding the development of patients with LUSC.

5. Conclusion

In our study, we screened for abnormal expression mRNA in early LUSC and performed KEGG analysis to identify the influence of abnormally expressed mRNA to the development of LUSC. The study can provide data for the early diagnosis and treatment of LUSC, and also provide a new research approach for finding potential therapeutic goals of LUSC.

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